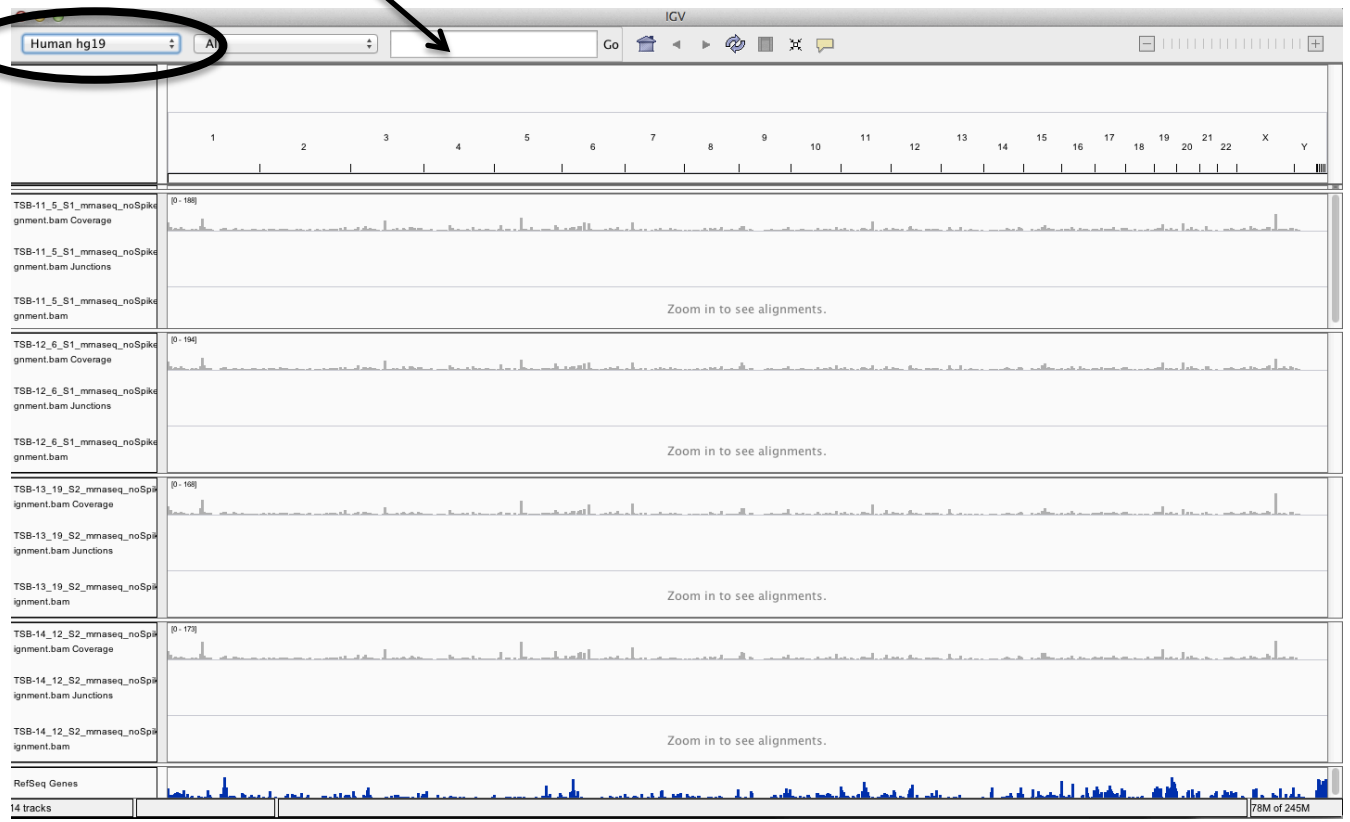
NGS read mapping : answers to questions

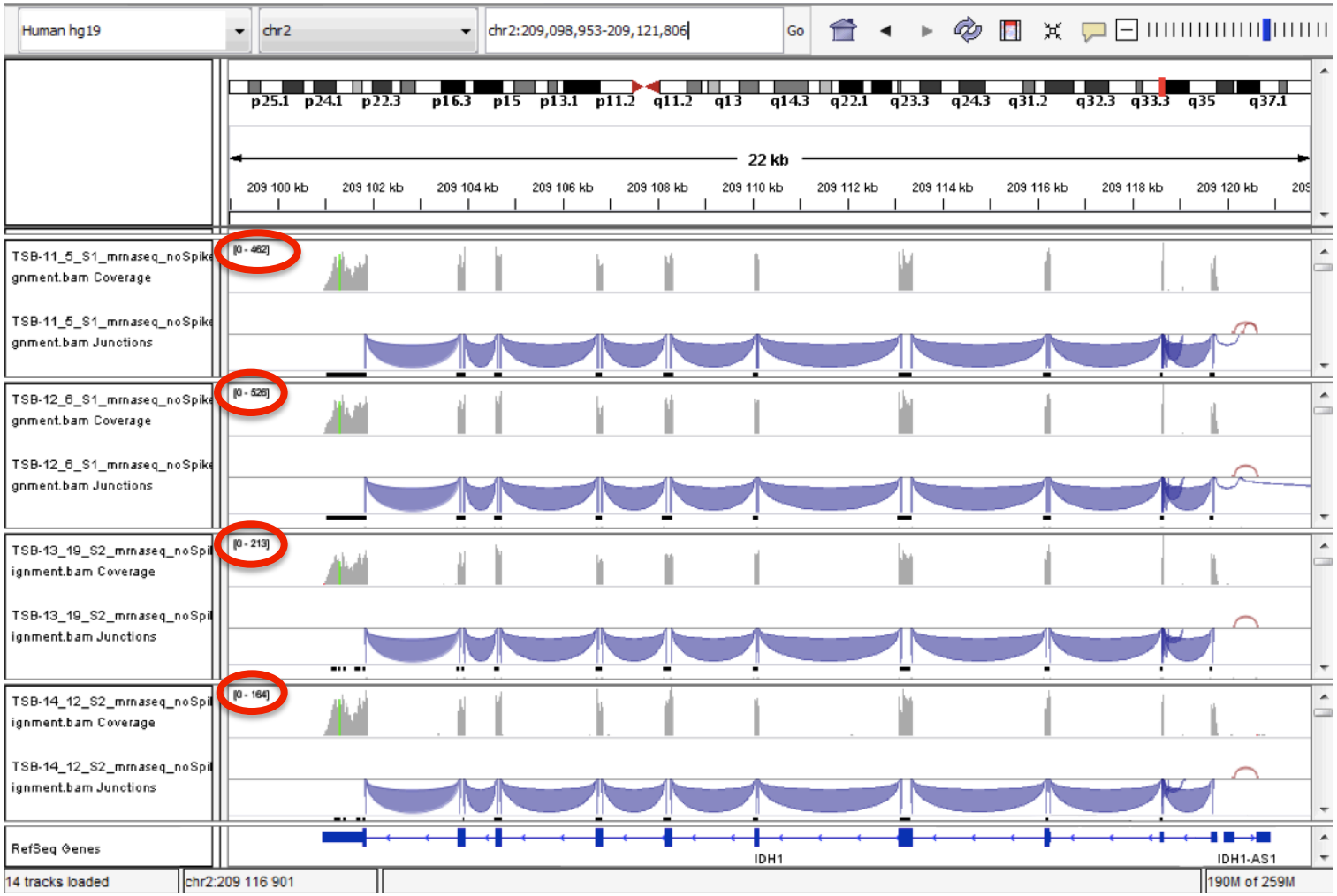
Céline Keime
keime@igbmc.fr

Question 1

- Genome assembly = hg19
- File → load from file and select the 4 BAM files
- Type IDH1 in the box and click Go

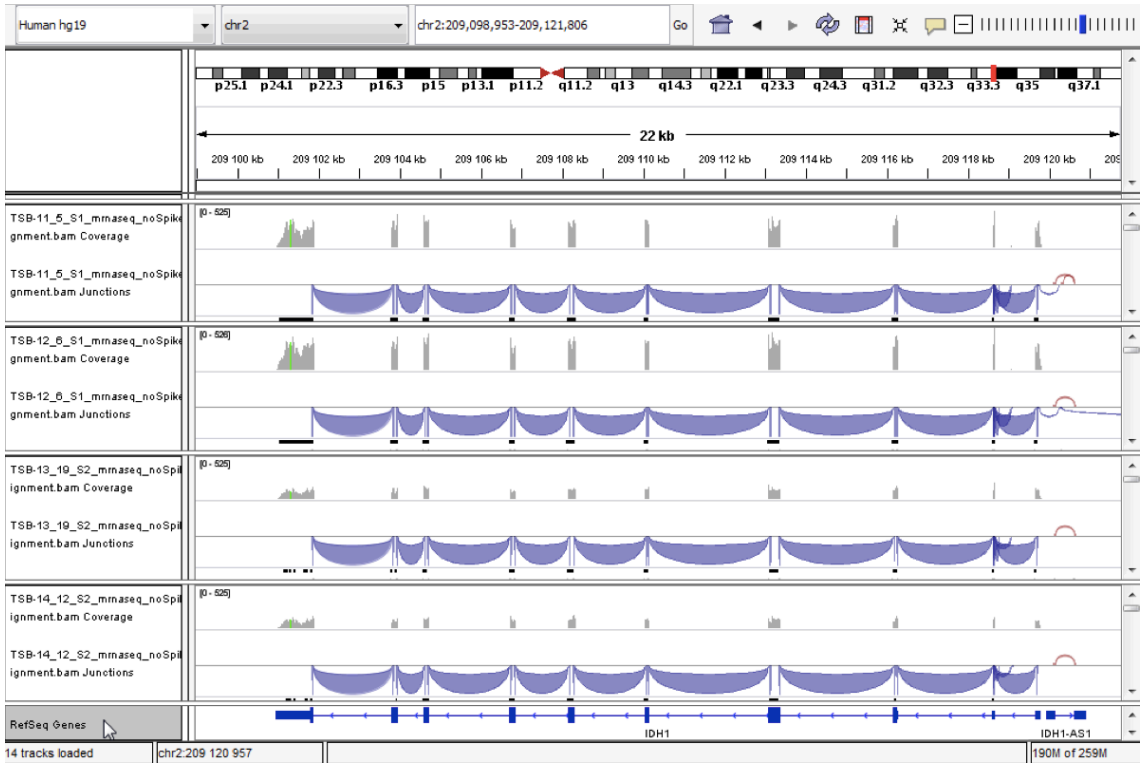


Question 1


Human hg19
chr2
chr2:209,098,953-209,121,806
Go
p25.1 p24.1 p22.3 p16.3 p15 p13.1 p11.2 q11.2 q13 q14.3 q22.1 q23.3 q24.3 q31.2 q32.3 q33.3 q35 q37.1
22 kb
209 100 kb 209 102 kb 209 104 kb 209 106 kb 209 108 kb 209 110 kb 209 112 kb 209 114 kb 209 116 kb 209 118 kb 209 120 kb
TSB-11_5_S1_mrnaseq_noSpike gnment.bam Coverage
[0 - 462]
TSB-11_5_S1_mrnaseq_noSpike gnment.bam Junctions
TSB-12_6_S1_mrnaseq_noSpike gnment.bam Coverage
[0 - 526]
TSB-12_6_S1_mrnaseq_noSpike gnment.bam Junctions
TSB-13_19_S2_mrnaseq_noSpil ignment.bam Coverage
[0 - 213]
TSB-13_19_S2_mrnaseq_noSpil ignment.bam Junctions
TSB-14_12_S2_mrnaseq_noSpil ignment.bam Coverage
[0 - 164]
TSB-14_12_S2_mrnaseq_noSpil ignment.bam Junctions
RefSeq Genes
IDH1
IDH1-AS1
14 tracks loaded
chr2:209 116 901
190M of 259M

Question 1

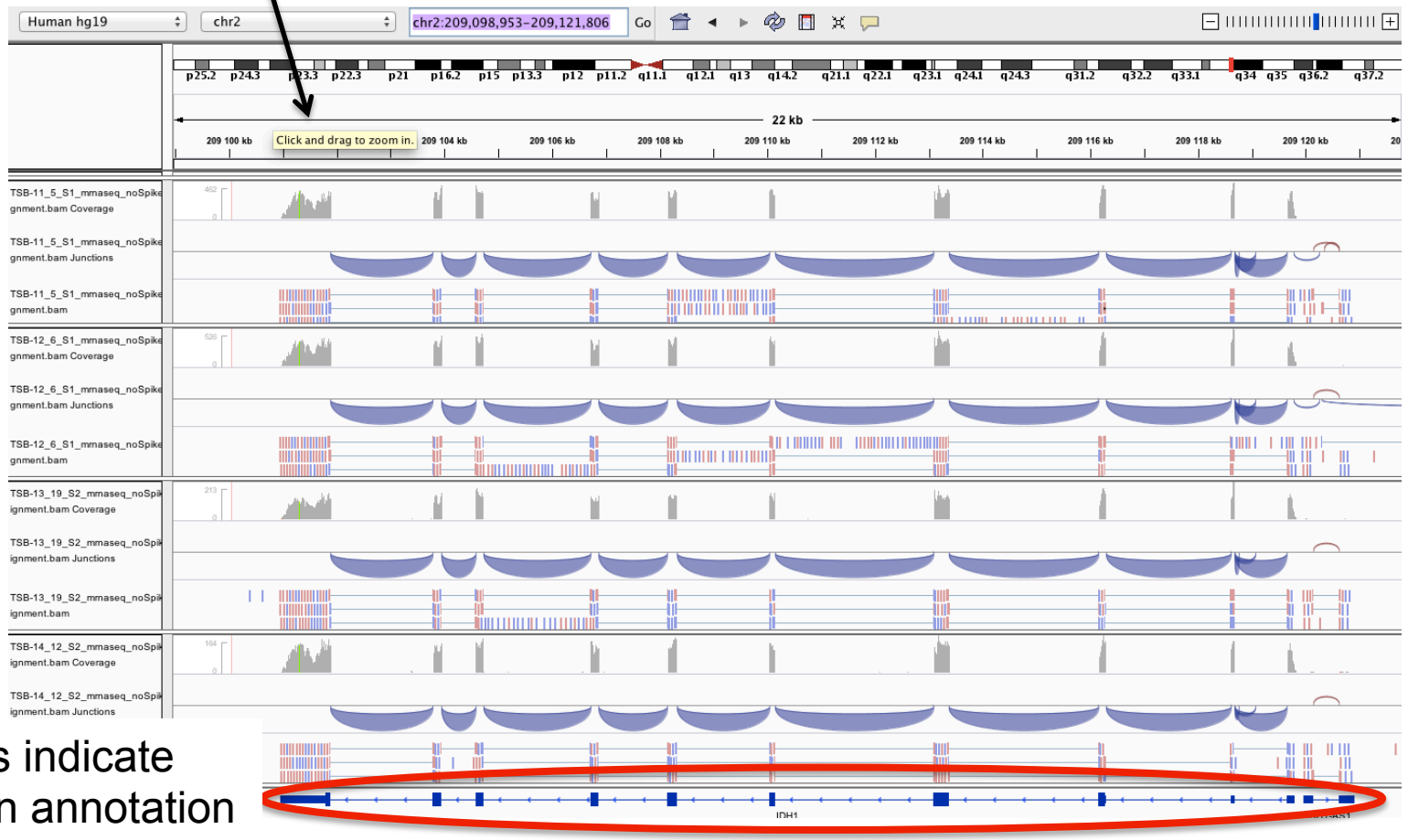
- Same scale for all tracks
  - Right-click on each coverage track → Set Data Range → Max = 525



- IDH1 is under-expressed in siMitf samples compared to siLuc ones
- More information on IDH1 → Click on gene name

Question 2

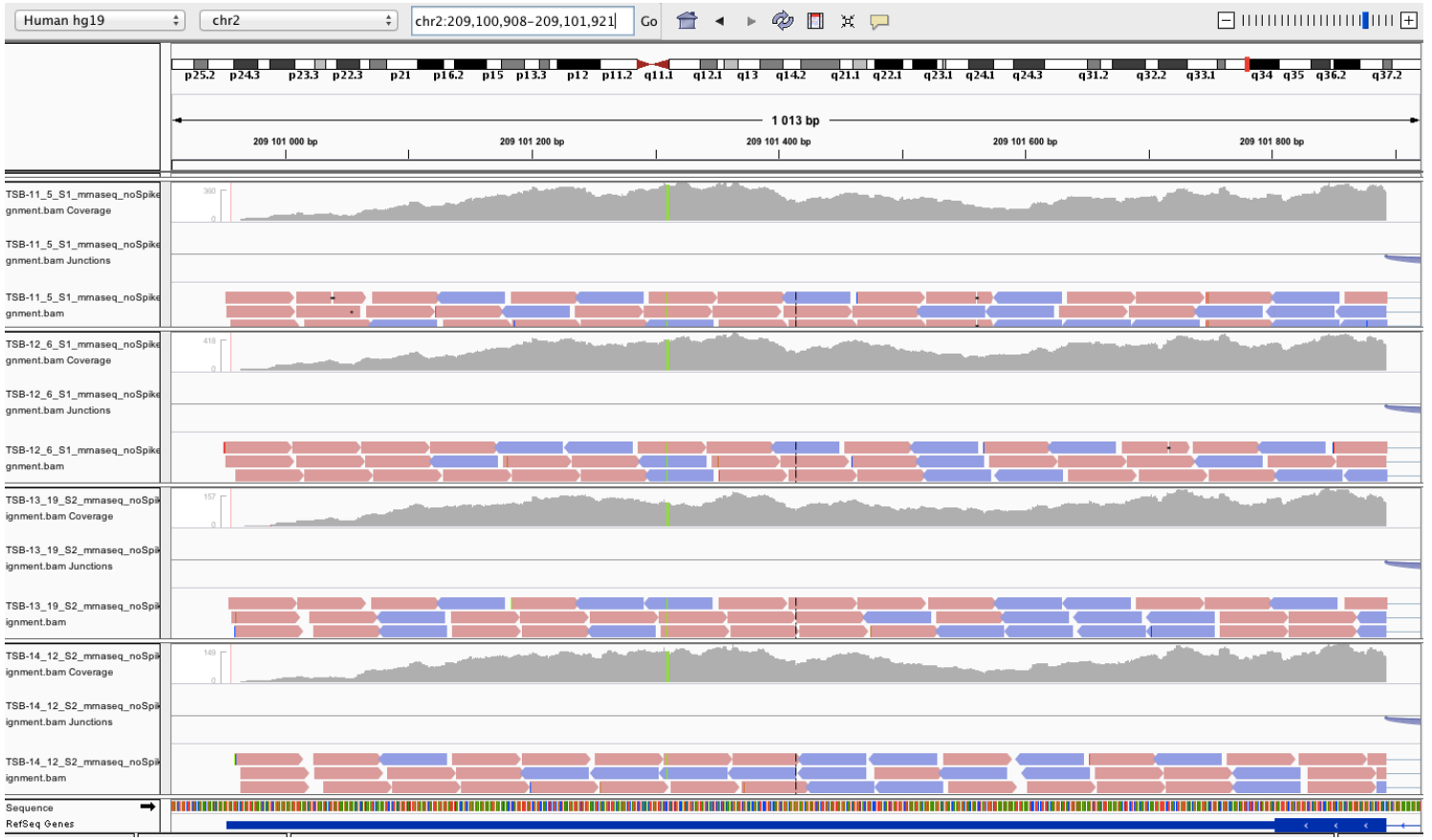
- Click and drag to define a window around the last exon in order to zoom in



Arrows indicate orientation annotation

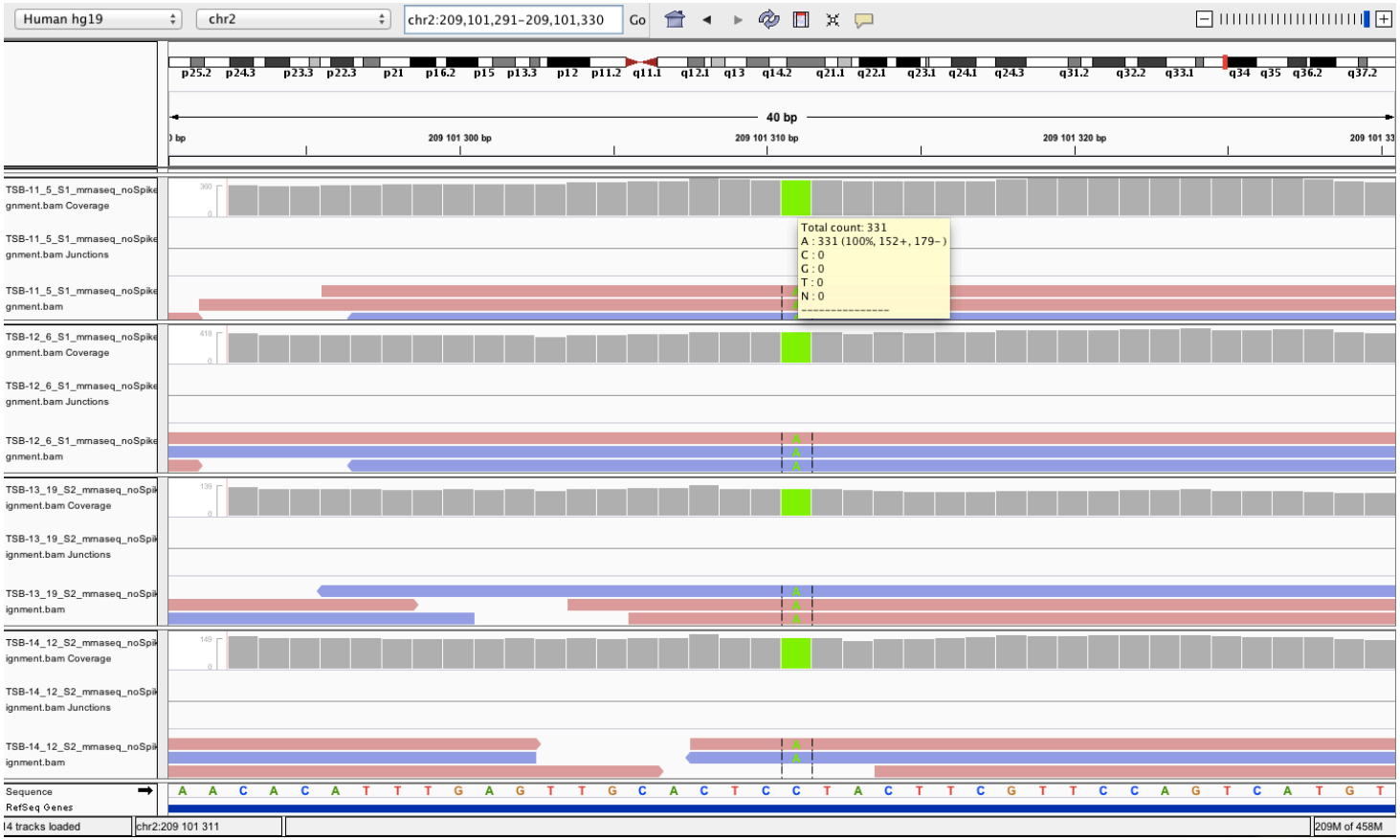
Question 2

- You can see the nucleotide difference in green
- Click and drag to zoom in around this position



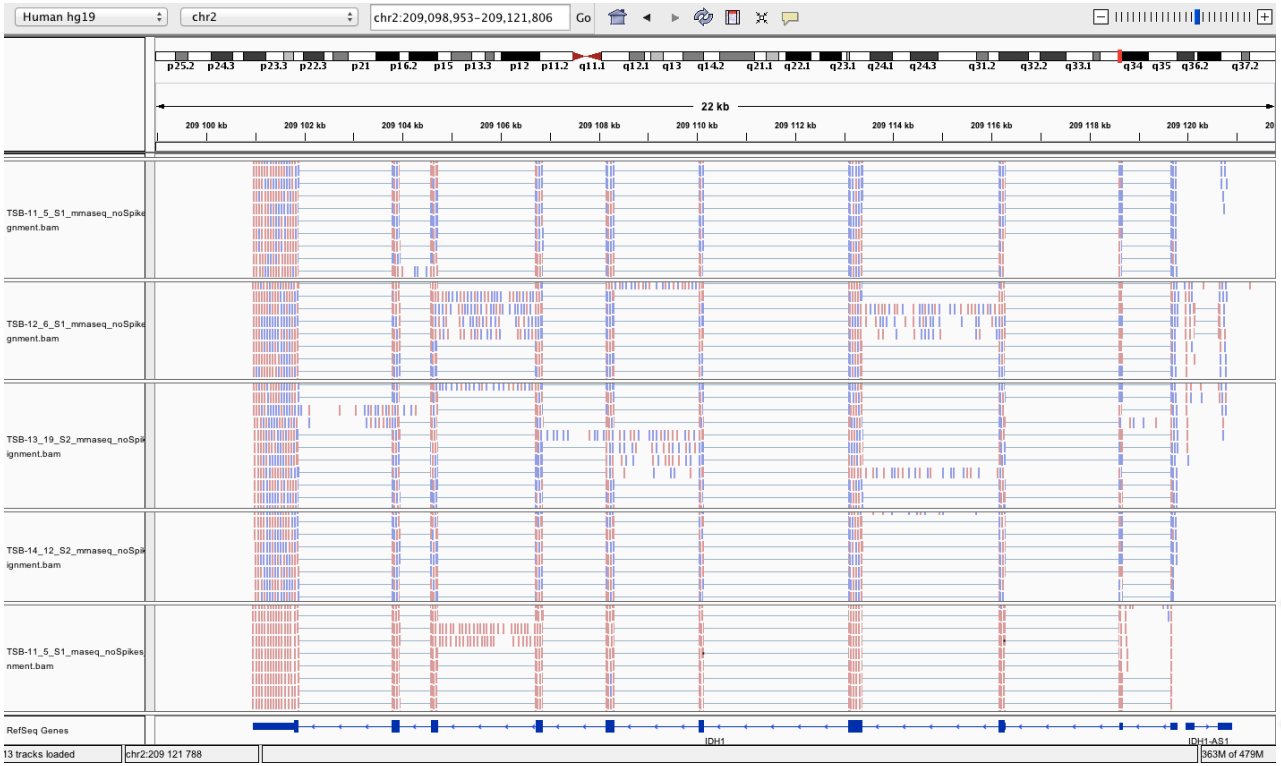
Question 2

- In the location chr2:209,101,311 :
  - A in 100% of the RNA-seq reads, C in the reference genome



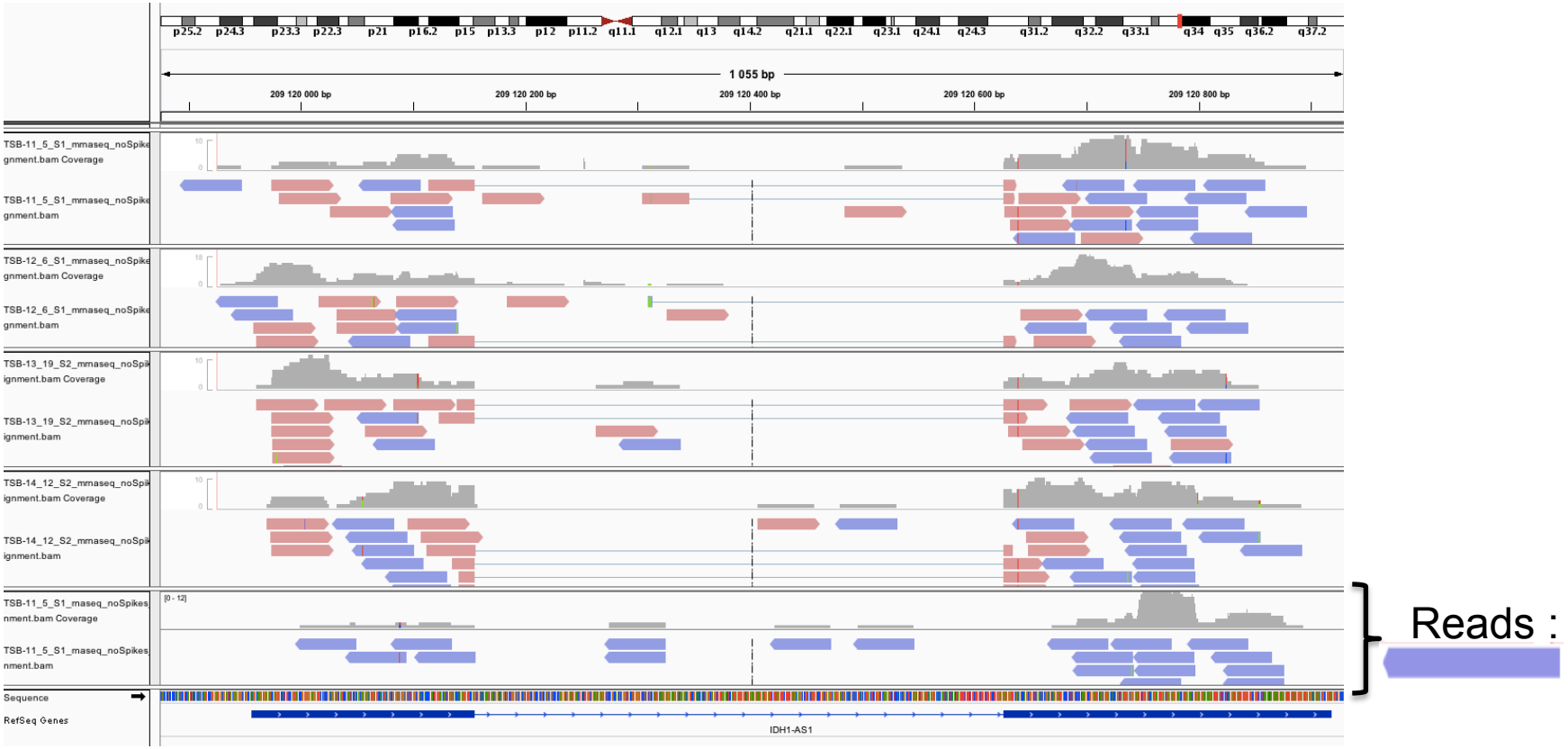
Question 3

- File → load from file and select TSB-11_5_S1_rnaseq_noSpikes_alignment.bam
- For each BAM file choose Color alignments by -> read strand
- e.g. IDH1 gene



Question 3

- e.g. IDH1-AS1 gene (IDH1 antisense RNA1)



- **The 2nd protocol is directional** (keep strand information)
  - All reads are in the opposite direction from the sense of transcription

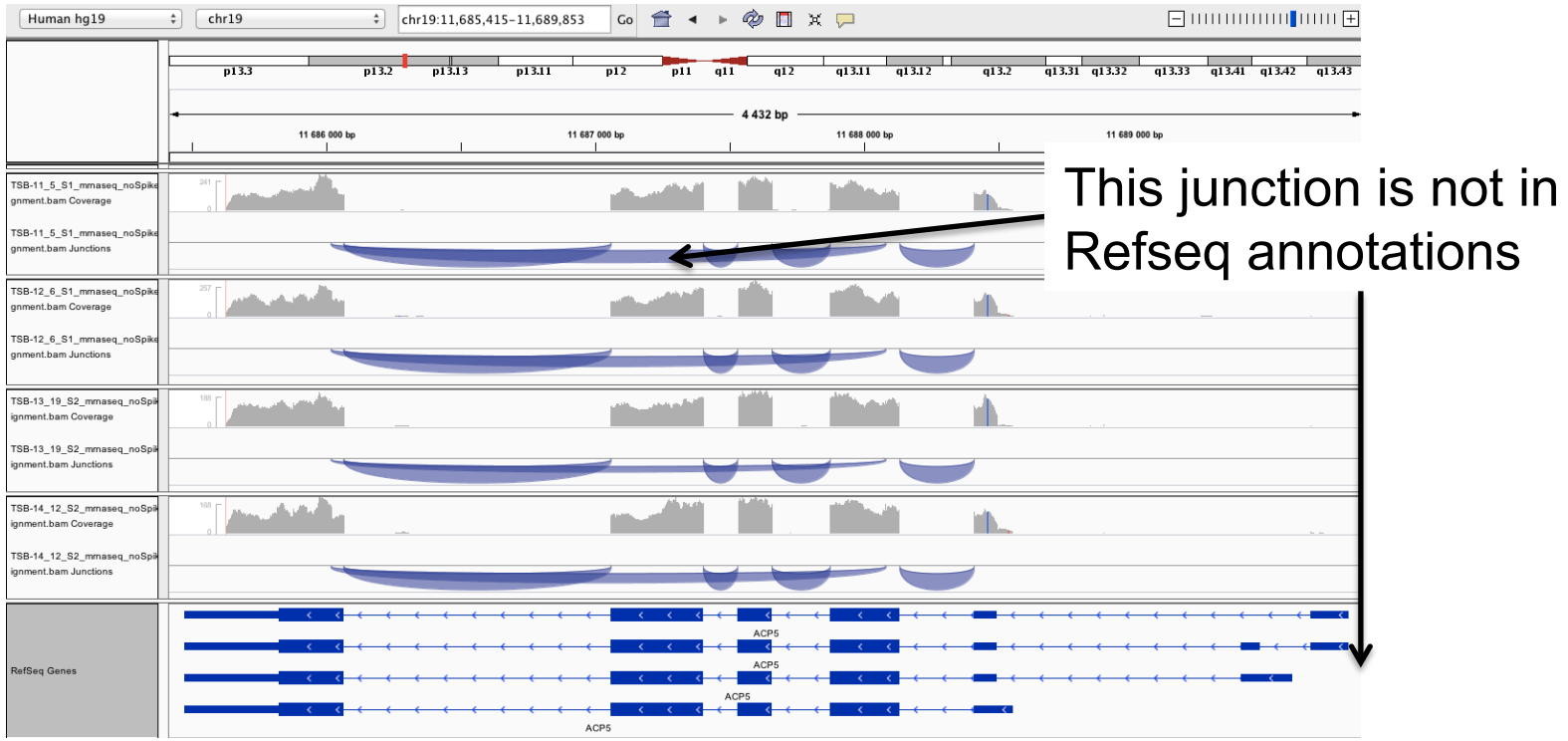
Question 4 : additional information

- Display of splice junctions
  - Strand
    - Blue junctions : + strand
    - Red junctions : - strand
  - Depth of coverage
    - The thickness of the arcs are proportional to the depth of coverage
    - All junctions with more than 50 reads have the same thickness
    - Hovering the mouse over a junction will display the coverage

Question 4

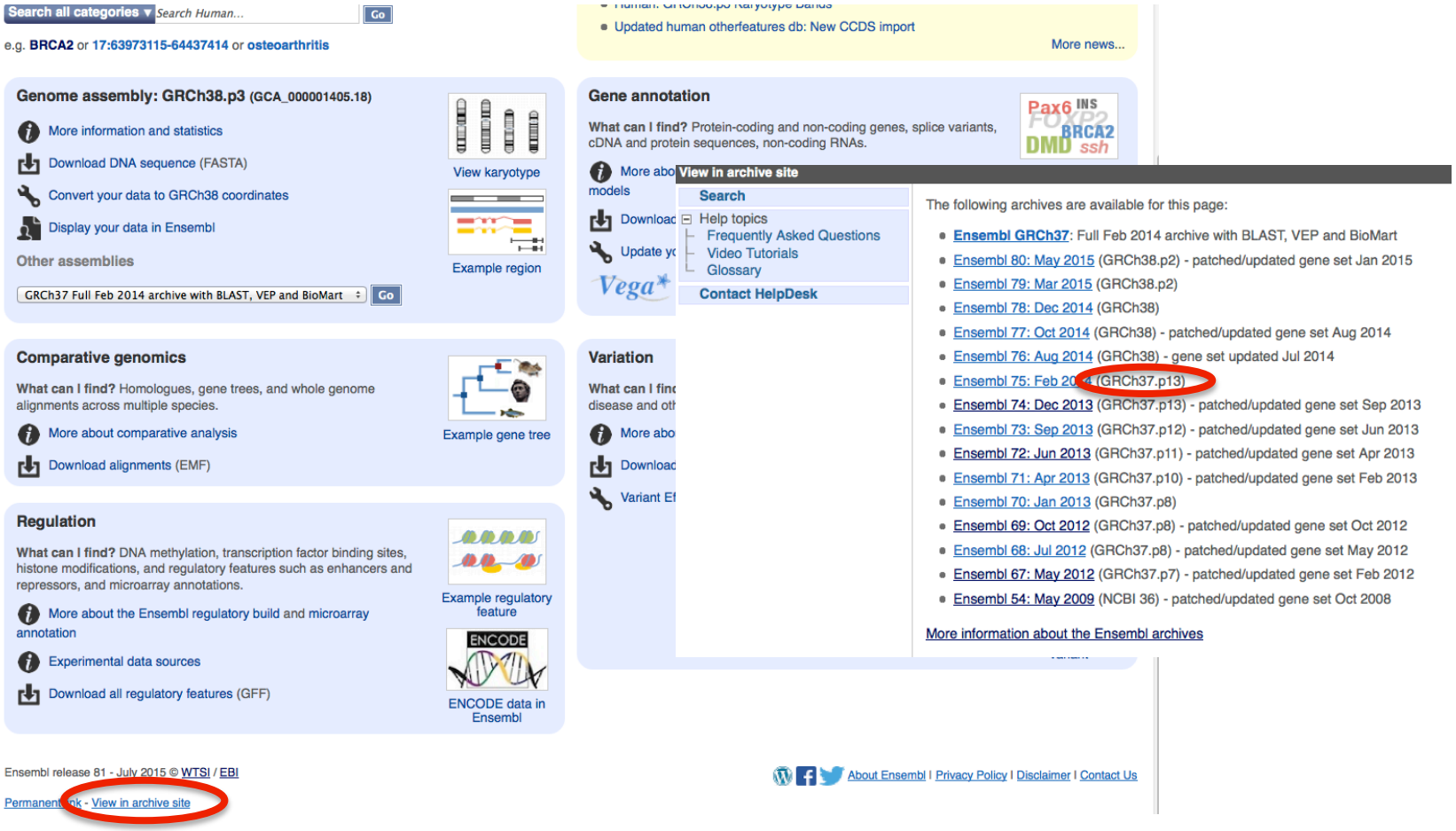
- View → preferences → Alignments tab → Splice junction track options panel.

  Choose Min flanking width=2 and Min junction coverage=10
- Right-click on the Refseq track → Expanded

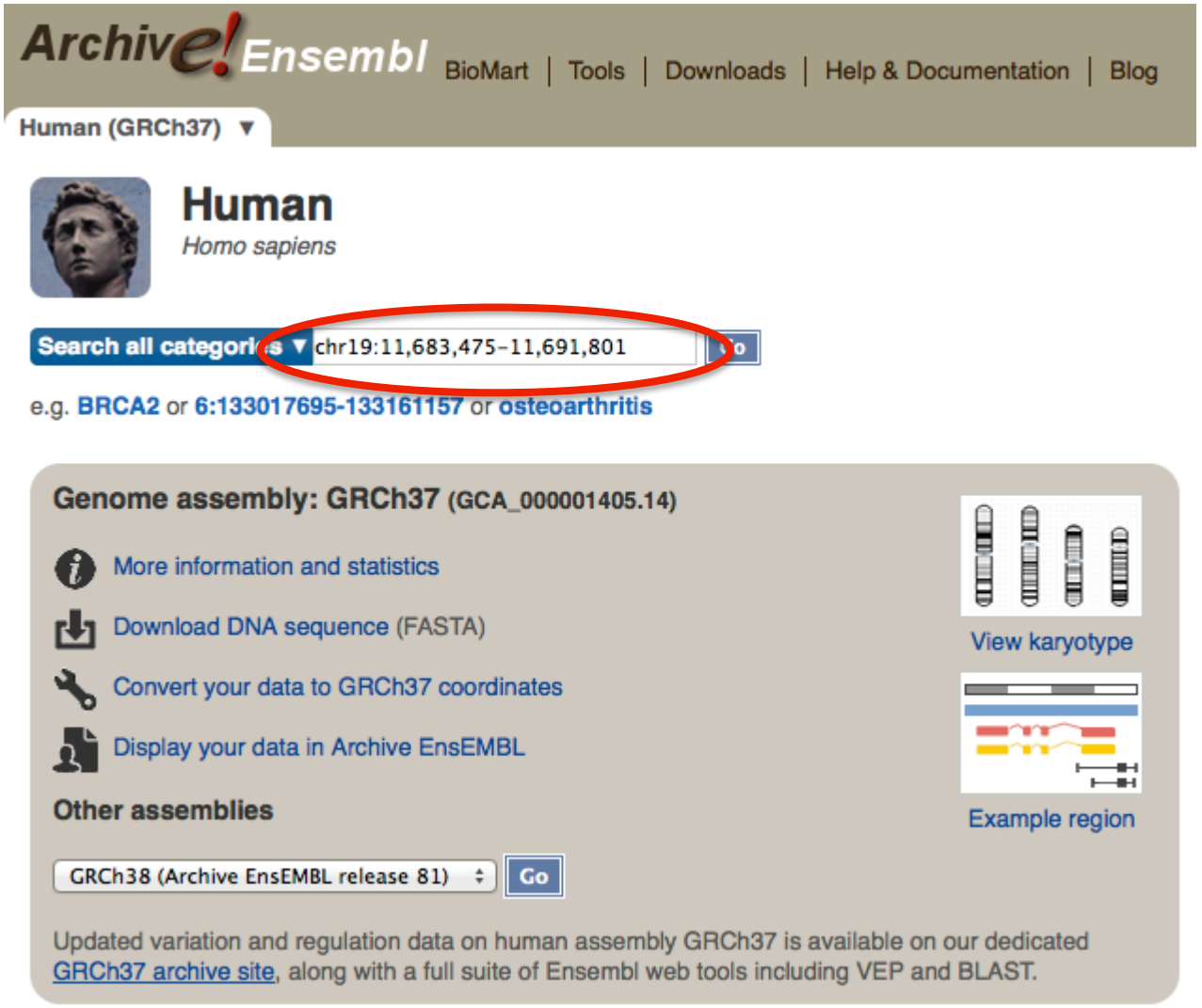


Question 4 : Ensembl

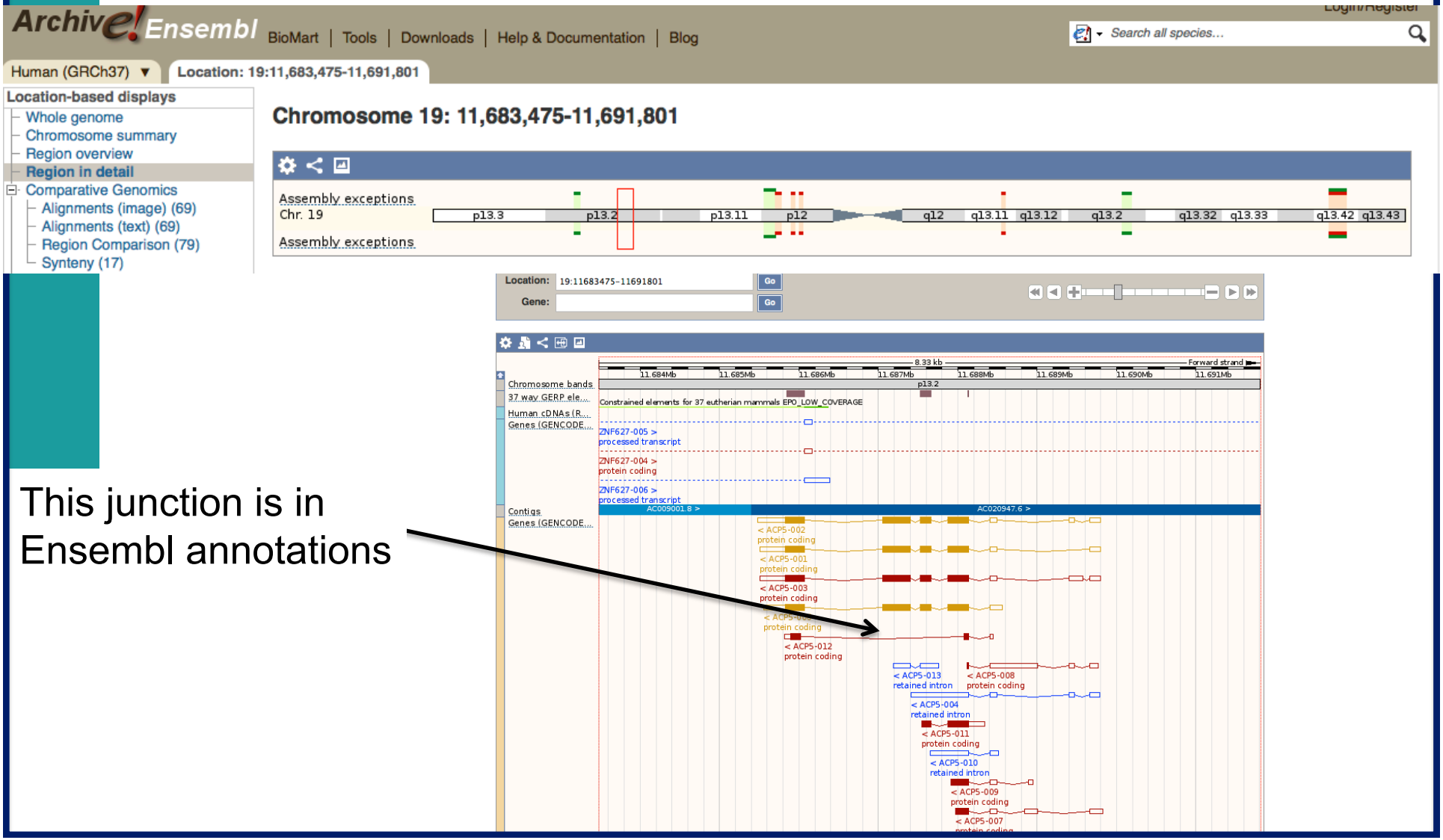
www.ensembl.org : choose an Ensembl version on hg19 (GRCh37)



Question 4 : Ensembl



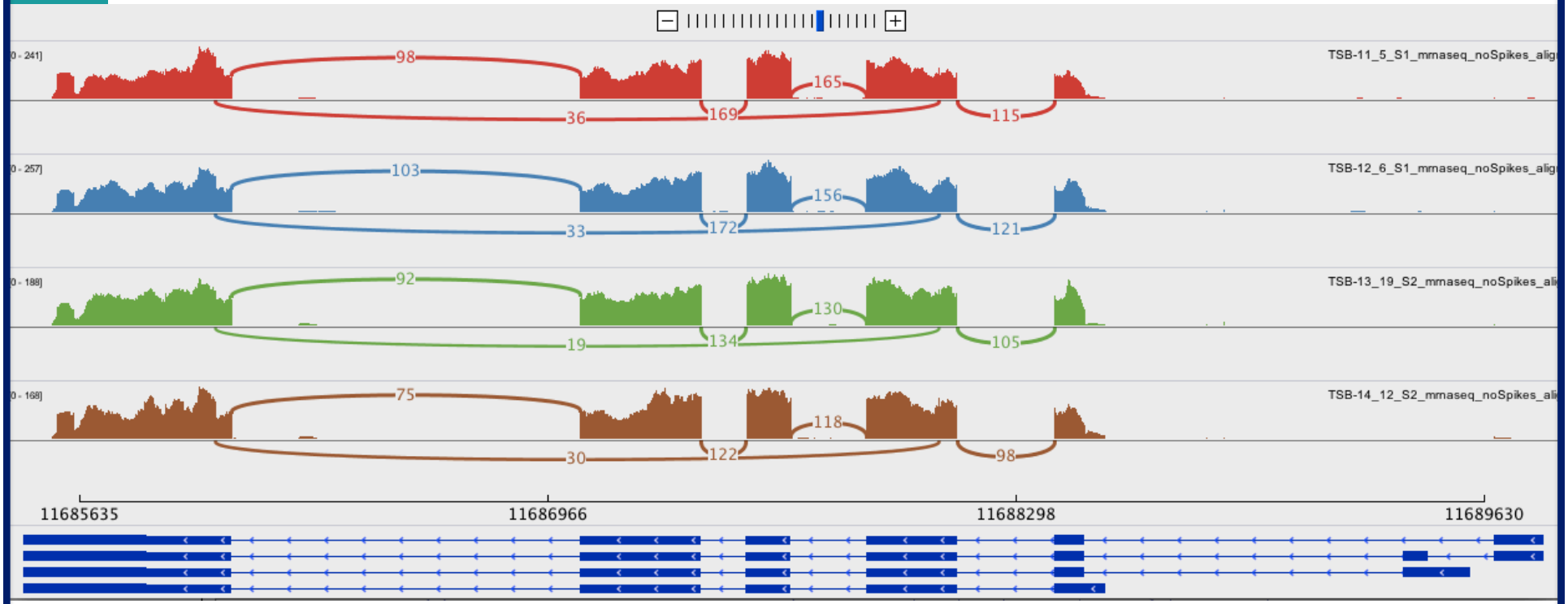
Question 4 : Ensembl



This junction is in Ensembl annotations

Question 4

- Shashimi plot



➔ Very useful to quickly screen differentially spliced exons along genomic regions of interest